Supplementary Information – Polymer-Tethered Ligand-Receptor Interactions between Surfaces. II

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APPENDIX A: POLYMER CONFINED BETWEEN TWO SURFACES

In this appendix we present in detail the calculations of the partition function of a polymer confined between surfaces with hard wall boundary conditions. We shall discuss two examples: gaussian chains with infinite extensions and rigid rods with finite extensions. But we first give a scaling analysis for a general chain model.

1. Scaling analysis

As discussed in the main text, polymer tethers have two effects: chain stretching and repulsion between the surfaces due to confinement. Both effects are classic problems in polymer physics. A systematic scaling analysis of polymers confined between surfaces can be found in Refs. [1] and [2]. Here we analyze the scaling of the size of the polymer tether in the presence of ligand-receptor binding. The scaling analysis is carried out for a polymer chain with Flory exponent ν, (i.e., $<R^2>\sim N^{2\nu}b$). The gaussian chain results follow by take $\nu = 1/2$.

When surfaces are far apart, the polymer chain is confined in a semi-infinite space, which gives the reference state for the problem. As surfaces come closer, ligand and the receptor groups at chain ends can meet and bind with each other. We assume that at this stage the two surfaces are still far apart so that the polymer chain is significantly stretched and chain confinement can be neglected, which will be justified *a posteriori*. In the strong stretching regime, the polymer chain can be viewed as a string of blobs of size ξ. Then the stretching energy is given by

$$E \sim k_{\rm B}T \times \text{number of blobs} \sim \frac{N}{(\xi/b)^{1/\nu}},$$

with the equilibrium end-to-end distance being equal to the surface separation,

$$L \sim N\xi^{1-\frac{1}{\nu}}b^{1/\nu}.$$

Therefore the stretching energy is given in terms of the end-to-end distance as

$$\frac{E}{k_{\rm B}T} \sim N\left(\frac{L}{Nb}\right)^{\frac{1}{1-\nu}} = \left(\frac{L}{N^{\nu}b}\right)^{\frac{1}{1-\nu}} \tag{A1}$$

When binding becomes possible, the molecular binding energy ϵ becomes comparable to the stretching energy, hence we have for the separation L_1 corresponding to the onset of binding

$$\left(\frac{L_1}{N^{\nu}b}\right)^{\frac{1}{1-\nu}} \sim \frac{\epsilon}{k_{\rm B}T} \Rightarrow L_1 \sim (\epsilon/k_{\rm B}T)^{1-\nu}N^{\nu}b. \tag{A2}$$

For $\epsilon \gg k_{\rm B}T$, $L_1 \gg N^{\nu}b$, and chain confinement is negligible, which justifies our assumption.

As surfaces come very close, the polymer chain is squeezed by the surfaces into a string of blobs on a plane parallel to the surfaces, with thickness L. Therefore the blob size is

$$\xi \simeq L,$$

and the free energy due to confinement is

$$V = k_{\rm B}T\frac{N}{(L/b)^{1/\nu}}. \tag{A3}$$

Putting these two terms together with the binding energy, the overall free energy of a single ligand-receptor pair is (C_1 and C_2 are dimensionless constants)

$$\frac{F}{k_{\rm B}T} = -f\left[\frac{\epsilon}{k_{\rm B}T} - C_1\left(\frac{L}{N^{\nu}b}\right)^{\frac{1}{1-\nu}}\right] + C_2\frac{N}{(L/b)^{1/\nu}}, \tag{A4}$$

and attains minimum at L_0 which is given by

$$L_0 \sim f^{-\nu(1-\nu)} N^\nu b, \tag{A5}$$

where f is the fraction of ligand-receptor bridges per ligand-receptor pair. For $\epsilon \gg k_\mathrm{B}T$, most molecules are bound ($f \approx 1$) at $L < L_1$, therefore the equilibrium separation between the surfaces is given by $L_0 \sim N^\nu b$.

The quenched case is more subtle. Assume $\epsilon \gg k_\mathrm{B}T$. The binding fraction of a tethered receptor is essentially the probability of finding a ligand within the "natural extension" of the receptor tether, $\rho_\mathrm{L}\left\langle r_\parallel^2 \right\rangle$. (Here "$\langle\rangle$" denotes the average over different chain conformations.) For a stretched chain, $r_\parallel^2$ is given by an ideal string of blobs in 2D,

$$\left\langle r_\parallel^2 \right\rangle \simeq \frac{N}{(\xi/b)^{1/\nu}} \xi^2 \sim N b^2 \left(\frac{N}{L}\right)^{\frac{2\nu-1}{1-\nu}}, \tag{A6a}$$

while for a confined chain the "natural" size of the tether parallel to the surface is that of a self-avoiding walk in 2D,

$$\left\langle r_\parallel^2 \right\rangle \simeq \left[\frac{N}{(\xi/b)^{1/\nu}}\right]^{2\nu_2} \xi^2 \sim N^{2\nu_2} L^{2-2\nu_2/\nu} b^{2\nu_2/\nu}, \tag{A6b}$$

ν_2 is the 2D Flory exponent.

For gaussian chains, $\nu = \nu_2 = 1/2$, the scaling is the same in both cases, $f \sim N b^2 \rho_\mathrm{L}$, which is a scaled density of the ligands. And we have [cf. Eq. (A5)]

$$L_0 \sim N^{1/2} b \left(\rho_\mathrm{L} N b^2\right)^{-1/4}. \tag{A7}$$

We see that in the quenched case the equilibrium occurs at a smaller surface separation compared with the annealed case. For swollen chains, $\nu \approx 3/5$ and $\nu_2 = 3/4$, the scaling in both scenarios (stretched and confined) also happen to be identical, and the probability of forming a ligand-receptor bridge is $f \sim \rho_\mathrm{L} N^{3/2} b^2 (L_z/b)^{-1/2}$. The extra $L_z^{-1/2}$ factor suggests that as surfaces get closer, the polymer tether extends further in the direction parallel to the surface (since $\nu_2 = 3/4 > \nu_3 \approx 3/5$). The scaled density is given by $\rho_\mathrm{L} N^{3/2} b^2$, and the equilibrium separation is [cf. Eq. (A5)]

$$L_0 \sim b N^{15/22} \left(\rho_\mathrm{L} N^{3/2} b^2\right)^{-3/11} \sim N^{6/22} \rho_\mathrm{L}^{-3/11}. \tag{A8}$$

Finally we can also estimate the interaction force between the surfaces due to binding. For a single bond, when the surfaces are pulled apart till the bond is broken, the total work done by the pulling force is roughly equal to the ϵ, hence we have (for binding fraction f) the average pulling force due to one ligand-receptor bond is

$$\tau \sim f \frac{\epsilon}{L_z^1 - L_z^0} \sim \frac{f k_\mathrm{B} T}{N^\nu b} \left(\frac{\epsilon}{k_\mathrm{B}T}\right)^\nu. \tag{A9}$$

2. Gaussian chain

First we consider gaussian chains. The Green's function of a free gaussian chain is governed by the partial differential equation [3]

$$\left(\frac{\partial}{\partial N} - \frac{b^2}{6}\nabla^2\right) G(\mathbf{r}, \mathbf{r}_0; N) = \delta^3(\mathbf{r} - \mathbf{r}_0)\delta(N). \tag{A10}$$

The non-adsorbing (hard wall) boundary conditions are

$$G(\mathbf{r}, \mathbf{r}_0; N) = 0 \text{ if } \mathbf{r} \text{ or } \mathbf{r}_0 \text{ is at the boundary.} \tag{A11}$$

The general result is

$$G(\mathbf{r}, \mathbf{r}_0; N) = g(x, x_0; N) g(y, y_0; N) g(z, z_0; N); \tag{A12}$$

$$g(x, x_0; N) = \frac{2}{L_x} \sum_{1 \le p \le \infty} \sin\left(\frac{p\pi x}{L_x}\right) \sin\left(\frac{p\pi x_0}{L_x}\right) \exp\left(-\frac{p^2 \pi^2 N b^2}{6 L_x^2}\right), \tag{A13}$$

and similar results for $g(y, y_0; N)$ and $g(z, z_0; N)$. Here (L_x, L_y, L_z) is the size of the box containing this polymer.

In our system the x and y directions are infinite, hence $g(x, x_0; N)$ and $g(y, y_0; N)$ are gaussian [$\mathbf{u} = (x, y)$]:

$$g(\mathbf{u}; N) = \frac{3}{2\pi N b^2} e^{-3u^2/2Nb^2}. \tag{A14}$$

In the z direction $g(z, z_0; N)$ is confined between 0 and L_z with hard-wall boundary conditions. (In the main text we use L to represent the surface separation, which is identical to L_z here.) The general expansion for $g(z, z_0; N)$ is

$$\begin{aligned} g(z, z_0; N) &= \sum_{-\infty \le k_z \le \infty} a(k_z) e^{ik_z(z_0 - z)} e^{-k_z^2 N b^2/6} \\ &= \sum_{0 \le k_z \le \infty} a(k_z) \left[\cos(k_z z_0)\cos(k_z z) + \sin(k_z z_0)\sin(k_z z)\right] e^{-k_z^2 N b^2/6}. \end{aligned}$$

To satisfy the Dirichlet boundary condition, we should choose $\sin(k_z z_0)\sin(k_z z)$; if we want a reflective boundary condition we should use $\cos(k_z z)\cos(k_z z_0)$.

For the non-adsorbing boundary condition we have

$$g(z, z_0; N) = \frac{2}{L_z} \sum_p \sin\frac{p\pi z}{L_z} \sin\frac{p\pi z_0}{L_z} \exp\left(-\frac{N b^2 p^2 \pi^2}{6 L_z^2}\right). \tag{A15}$$

In our problem one end of the polymer is anchored at z_0 very close to the surface, therefore the Green's function is given by

$$h_0(z; N) = \frac{2 z_0}{L_z^2} \sum_p p\pi \sin\frac{p\pi z}{L_z} \exp\left(-\frac{N b^2 p^2 \pi^2}{6 L_z^2}\right) \tag{A15$'$}$$

to first order in z_0/L_z.

The partition function is given by

$$q_z = \int_0^{L_z} dz h_0(z; N) = \frac{4 z_0}{L_z} \sum_{p=1,3,5,\ldots} e^{-p^2\pi^2/6l^2}, \tag{A16}$$

and is approximated by

$$q_z = \begin{cases} \dfrac{4 z_0}{L_z} e^{-\pi^2/6l^2} & l \ll 1, \\ \dfrac{\sqrt{6} z_0}{\sqrt{\pi N b^2}} & l \gg 1. \end{cases} \tag{A17}$$

Similarly for $h_0(L_z)$, the partition function of a ligand-receptor bridge we have[1]

$$\begin{aligned} h_0(L_z; N_\mathrm{L} + N_\mathrm{R}) := h_0(L_z - z_0; N_\mathrm{L} + N_\mathrm{R}) &= \frac{2}{L_z} \sum_p (-1)^{p+1} \sin\frac{p\pi z_0}{L_z} \sin\frac{p\pi z_0}{L_z} e^{-(p\pi)^2/6l^2} \\ &\approx \frac{2 z_0^2}{L_z^3} \sum_p (-1)^{p+1} (p\pi)^2 e^{-(p\pi)^2/6l^2} \\ &= -\frac{2 z_0^2}{L_z^3} \sum_p \cos p\pi (p\pi)^2 e^{-(p\pi)^2/6l^2}. \end{aligned} \tag{A18}$$

$h_0(L_z; N)$ can be approximated by

$$\begin{cases} \dfrac{2 z_0^2 \pi^2}{L_z^3} e^{-\pi^2/6l^2} & l \ll 1, \\ 18\sqrt{\dfrac{6}{\pi}} \dfrac{z_0^2}{(N b^2)^{3/2}} l^2 e^{-3l^2/2} & l \gg 1. \end{cases} \tag{A19}$$

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[1] Since $h_0(L_z)$ vanishes, we define it to be $h_0(L_z - z_0)$, as is shown later, for our interest this will not cause ambiguity.

Assembling the terms together we have

$$\begin{aligned} e^{\tilde{\epsilon}(l)-\epsilon} &= \sqrt{N}b \cdot \frac{h_0(L_z; N_\mathrm{L}+N_\mathrm{R})}{\int_0^{L_z} dz h_0(z; N_\mathrm{L}) \int_0^{L_z} dz h_0(z; N_\mathrm{R})} \\ &\approx \frac{1}{8l} \frac{\sum_p (-1)^{p+1} (p\pi)^2 e^{-(p\pi)^2/6l^2}}{\left[\sum_{p=2k-1} \exp\left(-\frac{N_\mathrm{L}}{N_\mathrm{L}+N_\mathrm{R}} \frac{p^2\pi^2}{6l^2}\right)\right] \cdot \left[\sum_{p=2k-1} \exp\left(-\frac{N_\mathrm{R}}{N_\mathrm{L}+N_\mathrm{R}} \frac{p^2\pi^2}{6l^2}\right)\right]}, \end{aligned} \tag{A20}$$

and the asymptotic limits are

$$\begin{cases} \dfrac{\pi^2}{8l} & l \ll 1, \\ 3\sqrt{6\pi} l^2 e^{-3l^2/2} \dfrac{\sqrt{N_\mathrm{L} N_\mathrm{R}}}{N_\mathrm{L}+N_\mathrm{R}} & l \gg 1. \end{cases} \tag{A21}$$

3. Rigid rod and variants

Here we study models with finite extensibility. First we consider a spherical chain model, in which the distribution of the free end is uniform within the hemisphere of radius R and zero outside. R can be identified as the contour length of the polymer, or as an approximation to the gaussian chain model, identified with the mean square end-to-end distance of the gaussian chain. For this model the Green's function of the polymer with one end fixed at the origin is given by

$$G(r, \theta, \phi; N) = \frac{3r^2 \sin\theta}{2\pi R^3}, \tag{A22}$$

and the partition function is

$$q = \begin{cases} 1 & L_z \geq R, \\ \frac{1}{3}\left[\frac{3L_z}{R} - \left(\frac{L_z}{R}\right)^3\right] & L_z < R. \end{cases} \tag{A23}$$

Slightly different is the model of a freely rotating rod, correspond to a short polymer whose contour length is smaller than the persistence length. The Green's function is

$$G(\mathbf{r}, \mathbf{r}_0; R) = \frac{1}{2\pi R^2} \delta\left(\frac{|\mathbf{r}-\mathbf{r}_0|}{R} - 1\right). \tag{A24}$$

R is the rod length, which is equal to the contour length of the polymer. For this model, the partition function is

$$q = \begin{cases} \frac{L_z}{R} & L_z < R, \\ 1 & L_z \geq R. \end{cases} \tag{A25}$$

The Green's function for the tether chain with two connected rods is conveniently represented by the length of the arc from the intersection circle of the two hemispheres spanned by the rod ends that is confined between the surfaces. The expression can be worked out, but is quite lengthy.

For ligand and receptor tethers we have $R_{\mathrm{L,R}} = N_{\mathrm{L,R}} b$, and the combined tether length is $(N_\mathrm{L} + N_\mathrm{R})b$. Let us define the scaled densities

$$\phi_{\mathrm{L,R}} = \rho_{\mathrm{L,R}} N^2 b^2.$$

From Eqs. (16) and (17) in Section IIB, the binding constant is given by

$$\begin{aligned} K =& \frac{\rho_\mathrm{LR}}{\rho_\mathrm{L}\rho_\mathrm{R}} = \frac{K_0 A \int_{\mathbf{r}_\mathrm{L},\mathbf{r}_\mathrm{R}} G_\mathrm{LR}(\mathbf{r}_\mathrm{L}, \mathbf{r}_\mathrm{R})}{\int_\mathbf{r} \int_{\mathbf{r}_\mathrm{L}} G(\mathbf{r}, \mathbf{r}_\mathrm{L}; N_\mathrm{L}) \int_\mathbf{r} \int_{\mathbf{r}_\mathrm{R}} G(\mathbf{r}, \mathbf{r}_\mathrm{R}; N_\mathrm{R})} \\ =& \frac{K_0}{q_\mathrm{L} q_\mathrm{R}} \int G_\mathrm{LR}(\mathbf{r}; N_\mathrm{L}, N_\mathrm{R}) \mathrm{d}^2\mathbf{r}. \end{aligned} \tag{A26}$$

G_LR is the partition function of a ligand-receptor bridge.

In the quenched case we have (cf. Appendix B for the definition of $w(\mathbf{r})$)

$$w(\mathbf{r}) = \frac{K_0 G_{\mathrm{LR}}(\mathbf{r}; N_{\mathrm{L}}, N_{\mathrm{R}})}{q_{\mathrm{L}} q_{\mathrm{R}}}. \tag{A27}$$

We immediately recognize from the finite extensibility that in these models binding is present only if $L_z \le (N_{\mathrm{L}}+N_{\mathrm{R}})b$. For the rigid-rod model, consider a ligand and a receptor with lateral separation $\mathbf{r}$, a necessary but not sufficient condition for binding to be possible is

$$|N_{\mathrm{L}} - N_{\mathrm{R}}|b \le \sqrt{\mathbf{r}^2 + L_z^2} \le (N_{\mathrm{L}} + N_{\mathrm{R}})b.$$

When surfaces come too close, binding becomes less probable.

APPENDIX B: LOW DENSITY EXPANSION FOR THE QUENCHED PROBLEM

For an immobile ligand anchored at $\mathbf{r}_{\mathrm{L}}$ and a receptor at $\mathbf{r}_{\mathrm{R}}$, the ratio of the Boltzmann factor of the bound state to that of the unbound state is given from Eqs. (16) and (17) to be

$$w(\mathbf{r}_{\mathrm{L}}, \mathbf{r}_{\mathrm{R}}) = \frac{q_{\mathrm{LR}}}{q_{\mathrm{L}} q_{\mathrm{R}}} = \frac{K_0 q^{\mathrm{t}}_{\mathrm{LR}}}{q^{\mathrm{t}}_{\mathrm{L}} q^{\mathrm{t}}_{\mathrm{R}}} = \frac{K_0 h(L_z) g(\mathbf{r}_{\mathrm{L}} - \mathbf{r}_{\mathrm{R}}; N_{\mathrm{L}} + N_{\mathrm{R}})}{q_{\mathrm{L}}(L_z) q_{\mathrm{R}}(L_z)}. \tag{B1}$$

Note that since molecules are immobile, the integration over $\mathbf{r}_{\mathrm{L}}$ or $\mathbf{r}_{\mathrm{R}}$ is removed; but the translational invariance implies that $w(\mathbf{r}_1, \mathbf{r}_2) = w(\mathbf{r}_1 - \mathbf{r}_2)$. Using $w(\mathbf{u})$ we can easily write down the first few terms of $F(m_{\mathrm{L}}, m_{\mathrm{R}})$ [cf. Eq. (25) in Section IIC]:

$$\begin{aligned}
-\beta F(1,1) &= \ln[1 + w(\mathbf{x}_1 - \mathbf{y}_2)] + \ln q_{\mathrm{L}} + \ln q_{\mathrm{R}}, \\
-\beta F(1,2) &= \ln q_{\mathrm{L}} + 2 \ln q_{\mathrm{R}} + \ln[1 + w(\mathbf{x}_1 - \mathbf{y}_1) + w(\mathbf{x}_1 - \mathbf{y}_2)], \\
-\beta F(2,2) &= 2 \ln q_{\mathrm{L}} + 2 \ln q_{\mathrm{R}} + \ln \left[1 + w(\mathbf{x}_1 - \mathbf{y}_1) + w(\mathbf{x}_1 - \mathbf{y}_2) + w(\mathbf{x}_2 - \mathbf{y}_1) + w(\mathbf{x}_2 - \mathbf{y}_2)\right. \\
&\quad \left. + w(\mathbf{x}_1 - \mathbf{y}_1) w(\mathbf{x}_2 - \mathbf{y}_2) + w(\mathbf{x}_1 - \mathbf{y}_2) + w(\mathbf{x}_2 - \mathbf{y}_1)\right].
\end{aligned}$$

(In this section we use $\beta = 1/k_{\mathrm{B}}T$.) Here $\mathbf{x}_{\mathrm{i}}$ and $\mathbf{y}_{\mathrm{j}}$ are positions of ligands and receptors, respectively.

Assuming that receptors and ligands are randomly distributed on the surfaces and for any receptor or ligand, its position distribution is independent of the others, we have the quenched average

$$\left\langle F(\mathbf{x}_1, \mathbf{x}_2, \cdots, \mathbf{x}_m, \mathbf{y}_1, \mathbf{y}_2, \cdots, \mathbf{y}_n) \right\rangle = \frac{1}{A^{i+j}} \int_{\{\mathbf{x}_{\mathrm{i}}\}, \{\mathbf{y}_{\mathrm{j}}\}} F(\{\mathbf{x}_{\mathrm{i}}\}, \{\mathbf{y}_{\mathrm{j}}\}),$$

Evaluating these averages is straightforward, which gives

$$-\beta \langle F(1,1) \rangle = \ln q_{\mathrm{L}} + \ln q_{\mathrm{R}} + \frac{1}{A} \int_{\mathbf{u}} \ln[1 + w(\mathbf{u})], \tag{B2a}$$

$$-\beta \langle F(1,2) \rangle = \ln q_{\mathrm{L}} + 2 \ln q_{\mathrm{R}} + \frac{1}{A^2} \int_{\mathbf{u}_1, \mathbf{u}_2} \ln[1 + w(\mathbf{u}_1) + w(\mathbf{u}_2)], \tag{B2b}$$

$$-\beta \langle F(2,1) \rangle = 2 \ln q_{\mathrm{L}} + \ln q_{\mathrm{L}} + \frac{1}{A^2} \int_{\mathbf{u}_1, \mathbf{u}_2} \ln[1 + w(\mathbf{u}_1) + w(\mathbf{u}_2)], \tag{B2c}$$

$$-\beta \langle F(1,m) \rangle = \ln q_{\mathrm{L}} + m \ln q_{\mathrm{R}} + \frac{1}{A^m} \int_{\mathbf{u}_1, \cdots \mathbf{u}_m} \ln[1 + \sum_m w(\mathbf{u}_m)], \tag{B2d}$$

$$\begin{aligned}
-\beta \langle F(2,2) \rangle &= 2 \ln q_{\mathrm{L}} + 2 \ln q_{\mathrm{R}} + \frac{1}{A^3} \int_{\mathbf{u}_1, \mathbf{u}_2, \mathbf{v}_1} \ln[1 + w(\mathbf{u}_1) + w(\mathbf{u}_2) + w(\mathbf{u}_1 + \mathbf{v}) + w(\mathbf{u}_2 + \mathbf{v}) \\
&\quad + w(\mathbf{u}_2) w(\mathbf{u}_1 + \mathbf{v}) + w(\mathbf{u}_1) w(\mathbf{u}_2 + \mathbf{v})].
\end{aligned} \tag{B2e}$$

Substituting these back into Eq. (25) we have

$$-\beta \bar{F}^{(1,1)} = \frac{1}{e^{A\rho_{\mathrm{L}} + A\rho_{\mathrm{R}}}} \sum_{m_{\mathrm{L}} \ge 1, m_{\mathrm{R}} \ge 1} \frac{(A\rho_{\mathrm{L}})^{m_{\mathrm{L}}} (A\rho_{\mathrm{R}})^{m_{\mathrm{R}}}}{m_{\mathrm{L}}! m_{\mathrm{R}}!} \frac{m_{\mathrm{L}} m_{\mathrm{R}}}{A} \int_{\mathbf{u}} \ln[1 + w(\mathbf{u})]$$

$$= A\rho_{\rm L}\rho_{\rm R}\int_{\mathbf{u}} \ln[1+w(\mathbf{u})] \tag{B3a}$$
$$= A\rho_{\rm L}\rho_{\rm R}\mathcal{F}^{(1,1)};$$
$$-\beta\bar{F}^{(1,2)} = \frac{1}{e^{A(\rho_{\rm L}+\rho_{\rm R})}}\sum_{m_{\rm L}\geq 1, m_{\rm R}\geq 2}\frac{(A\rho_{\rm L})^{m_{\rm L}}(A\rho_{\rm R})^{m_{\rm R}}}{m_{\rm L}!m_{\rm R}!}m_{\rm L}^1 m_{\rm R}^2$$
$$\left\{\frac{1}{A^2}\int_{\mathbf{u}_1,\mathbf{u}_2}\ln[1+w(\mathbf{u}_1)+w(\mathbf{u}_2)] - \frac{2}{A}\int_{\mathbf{u}}\ln[1+w(\mathbf{u})]\right\}$$
$$= \frac{A\rho_{\rm L}\rho_{\rm R}^2}{2}\int_{\mathbf{u}_1,\mathbf{u}_2}\{\ln[1+w(\mathbf{u}_1)+w(\mathbf{u}_2)] - \ln[1+w(\mathbf{u}_1)] - \ln[1+w(\mathbf{u}_2)]\} \tag{B3b}$$
$$= \frac{A\rho_{\rm L}\rho_{\rm R}^2}{2}\mathcal{F}^{(1,2)};$$
$$-\beta\bar{F}^{(1,m)} = \frac{A\rho_{\rm L}\rho_{\rm R}^m}{m!}\left\{\int_{\mathbf{u}_1,\mathbf{u}_2,\cdots\mathbf{u}_m}\ln\left[1+\sum_i w(\mathbf{u}_i)\right] - \sum_{1\leq k<m}A^{m-k}{\rm C}_m^k\mathcal{F}^{(1,k)}\right\}; \tag{B3c}$$
$$-\beta\bar{F}^{(2,2)} = \frac{A\rho_{\rm L}^2\rho_{\rm R}^2}{4}\left(\int_{\mathbf{u}_1,\mathbf{u}_2,\mathbf{v}}\ln\Big[1+w(\mathbf{u}_1)+w(\mathbf{u}_2)+w(\mathbf{u}_1+\mathbf{v})+w(\mathbf{u}_2+\mathbf{v})\right.$$
$$\left.+\,w(\mathbf{u}_1)w(\mathbf{u}_2+\mathbf{v})+w(\mathbf{u}_2)w(\mathbf{u}_1+\mathbf{v})\Big]\right.$$
$$-\,4A\int_{\mathbf{u}_{\rm L},\mathbf{u}_{\rm R}}\Big\{\ln\big[1+w(\mathbf{u}_1)+w(\mathbf{u}_2)\big]-\ln[1+w(\mathbf{u}_1)]-\ln[1+w(\mathbf{u}_2)]\Big\}$$
$$\left.-\,4A^2\int_{\mathbf{u}}\ln[1+w(\mathbf{u})]\right) \tag{B3d}$$
$$= \frac{A\rho_{\rm L}^2\rho_{\rm R}^2}{4}\mathcal{F}^{(2,2)}.$$

For gaussian chains, the quantity $w(\mathbf{r})$ can be rewritten as

$$w(\mathbf{u}) = \frac{K_0 h_0(l)}{q_{\rm L}(l)q_{\rm R}(l)}g(\mathbf{u}; N_{\rm L}+N_{\rm R}) = \frac{3}{2\pi}\exp\left[\beta\tilde{\epsilon}(l) - \frac{3u^2}{2Nb^2}\right], \tag{B4}$$

where the effective binding energy $\tilde{\epsilon}$ is defined above in Eqs. (21)–(23) and the second term accounts for lateral stretching. From Eq. (B4) we see that (a) the effective binding energy has a similar dependence on the surface separation as in the annealed case as reflected in $\tilde{\epsilon}$; (b) each integral over $\mathbf{u}$ gives a factor of Nb^2, hence

$$\mathcal{F}^{(n,m)} \propto (Nb^2\rho)^{n+m-1},$$

and we see that in Eq. (B3) the real expansion parameter is $\phi = \rho N b^2$. (Similarly one can verify that in the case of rigid rods, the expansion is in terms of $\phi = \rho N^2 b^2$.) For large binding energy $\beta\tilde{\epsilon} \gg 1$, each integral over the scaled $\mathbf{u}$ also contributes a factor of $\beta\tilde{\epsilon}$, therefore the asymptotic density expansion is valid only if

$$\beta\tilde{\epsilon}\phi \ll 1.$$

The density of bound pairs is obtained by taking the derivative of $\bar{F}$ against $\ln w$. At the leading order the density of bound pairs can be expressed in a close form:

$$\bar{\rho}_{\rm LR} = \rho_{\rm L}\rho_{\rm R}\int_{\mathbf{u}}\frac{\mathrm{d}}{\mathrm{d}\ln w}\ln(1+w) = \int_{\mathbf{r}}\frac{w(\mathbf{u})}{1+w(\mathbf{u})}, \tag{B5}$$

which for gaussian chain with

$$w(\mathbf{u}) \propto e^{-\frac{3u^2}{2}},$$

becomes

$$\bar{\rho}_{\rm LR} = 2\pi\rho_{\rm L}\rho_{\rm R}\int_0^\infty \frac{w(0)e^{-\frac{3u^2}{2}}}{1+w(0)e^{-\frac{3u^2}{2}}}u\mathrm{d}u = \frac{2\pi}{3}\rho_{\rm L}\rho_{\rm R}Nb^2\ln(1+w(0)). \tag{B6}$$

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